V. Ford
65290

```
=> fil medl,caplus,biosis,embase,wpids,jicst;s (antibacterial agents/ct or
bactericide? or disinfectant? or antiseptic? or antibacterial agent)
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       4.16       4.31

FILE 'MEDLINE' ENTERED AT 14:22:56 ON 13 AUG 2001

FILE 'CAPLUS' ENTERED AT 14:22:56 ON 13 AUG 2001
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FILE 'JICST-EPLUS' ENTERED AT 14:22:56 ON 13 AUG 2001
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L2        10969 FILE MEDLINE
L3        79207 FILE CAPLUS
L4        17891 FILE BIOSIS
L5         9816 FILE EMBASE
L6        25587 FILE WPIDS
L7         4670 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L8       148140 (ANTIBACTERIAL AGENTS/CT OR BACTERICIDE? OR DISINFECTANT? OR
                ANTISEPTIC? OR ANTIBACTERIAL AGENT)

=> s l8 and (non pathogen? or nonpathogen?)
L9           14 FILE MEDLINE
L10          62 FILE CAPLUS
L11          18 FILE BIOSIS
L12          15 FILE EMBASE
L13          28 FILE WPIDS
L14           4 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L15         141 L8 AND (NON PATHOGEN? OR NONPATHOGEN?)

=> s l15 and (escherchia or lactobacill? or lactococ? orbifidobacter? or
eubacter? or salmonella or propionobacter or shigella or campylobacter or
gardnerella or mycobacter? or pseudomonas or staphyloco?)
L16           4 FILE MEDLINE
<------User Break------>
u
SEARCH ENDED BY USER
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Prepared by M. Hale 308-4258

=> s l15 and (escherchia or lactobacill? or lactococ? or bifidobacter? or eubacter? or salmonella or propionobacter or shigella or campylobacter or gardnerella or mycobacter? or pseudomonas or staphyloco?)
L18 4 FILE MEDLINE
L19 28 FILE CAPLUS
L20 9 FILE BIOSIS
L21 5 FILE EMBASE
L22 5 FILE WPIDS
L23 2 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L24 53 L15 AND (ESCHERCHIA OR LACTOBACILL? OR LACTOCOC? OR BIFIDOBACTER
? OR EUBACTER? OR SALMONELLA OR PROPIONOBACTER OR SHIGELLA OR CAMPYLOBACTER OR GARDNERELLA OR MYCOBACTER? OR PSEUDOMONAS OR STAPHYLOCO?)

=> s l24 and (plasmid? or rk2 or r6k or pcu1 or p15a or pip501 or pam(w)(b or beta)(w)1 or pcrg1600 or rsf1010)
L25 0 FILE MEDLINE
L26 0 FILE CAPLUS
L27 0 FILE BIOSIS
L28 0 FILE EMBASE
L29 0 FILE WPIDS
L30 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L31 0 L24 AND (PLASMID? OR RK2 OR R6K OR PCU1 OR P15A OR PIP501 OR PAM(W)(B OR BETA)(W) 1 OR PCRG1600 OR RSF1010)

=> s l24 and transmiss?
L32 1 FILE MEDLINE
L33 0 FILE CAPLUS
L34 0 FILE BIOSIS
L35 0 FILE EMBASE
L36 0 FILE WPIDS
L37 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L38 1 L24 AND TRANSMISS?

=> d cbib abs hit

L38 ANSWER 1 OF 1 MEDLINE
91129375 Document Number: 91129375. PubMed ID: 2126472. AIDS: practising safe endoscopy. Hanson P J. BAILLIERES CLINICAL GASTROENTEROLOGY, (1990 Jun) 4 (2) 477-94. Ref: 82. Journal code: BBG; 8704786. ISSN: 0950-3528. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The emergence of HIV has provoked a widespread reappraisal of infection control practices in endoscopy units. Infection control practices should be applied to all patients alike without recourse to selection or screening. Although there has only been one reported instance of viral **transmission** at endoscopy, HIV could in theory be transmitted by a contaminated endoscope. Experience suggests that this is more likely to

occur from damaged endoscopes, if an unsuitable **disinfectant** is used or endoscopes are not precleaned. In-use studies have shown that HIV contaminates endoscopes used on patients with AIDS, but in amounts too small to cause infection in tissue cultures. Cleaning in neutral detergent is extremely effective in removing contaminating micro-organisms, including HIV, from endoscopes. Aldehydes are the **disinfectants** of choice, but any **disinfectant** may fail if organic material is not removed by cleaning. After thorough cleaning, short disinfection times (e.g. four minutes) ensure inactivation of all relevant micro-organisms except Cryptosporidium and **mycobacteria**, although in practice even these organisms are likely to be reduced to **non-pathogenic** levels. Accidental needlestick injuries are the greatest hazard in the endoscopy suite; needles should not be resheathed and biopsy forceps must be handled with great care. The wearing of gloves should become second nature.

AB The emergence of HIV has provoked a widespread reappraisal of infection control practices in endoscopy units. Infection control practices should be applied to all patients alike without recourse to selection or screening. Although there has only been one reported instance of viral **transmission** at endoscopy, HIV could in theory be transmitted by a contaminated endoscope. Experience suggests that this is more likely to occur from damaged endoscopes, if an unsuitable **disinfectant** is used or endoscopes are not precleaned. In-use studies have shown that HIV contaminates endoscopes used on patients with AIDS, but in amounts too small to cause infection in tissue cultures. Cleaning in neutral detergent is extremely effective in removing contaminating micro-organisms, including HIV, from endoscopes. Aldehydes are the **disinfectants** of choice, but any **disinfectant** may fail if organic material is not removed by cleaning. After thorough cleaning, short disinfection times (e.g. four minutes) ensure inactivation of all relevant micro-organisms except Cryptosporidium and **mycobacteria**, although in practice even these organisms are likely to be reduced to **non-pathogenic** levels. Accidental needlestick injuries are the greatest hazard in the endoscopy suite; needles should not be resheathed and biopsy forceps must be handled with great care. The wearing of gloves should become second nature.

CT Check Tags: Human
Acquired Immunodeficiency Syndrome: CO, complications
Acquired Immunodeficiency Syndrome: PC, prevention & control
***Acquired Immunodeficiency Syndrome: TM, transmission**
Cross Infection: CO, complications
Cross Infection: PC, prevention & control
***Cross Infection: TM, transmission**
Disinfection: MT, methods
Disinfection: ST, standards
*Endoscopy: AE, adverse effects
Endoscopy: ST, standards
*Equipment Contamination
Gastrointestinal Diseases: CO, complications
*Gastrointestinal Diseases: DI, diagnosis
Glutaral: AE, adverse effects

Prepared by M. Hale 308-4258

Glutaral: TU, therapeutic use
Occupational Exposure

```
=> s (atimicrobial or anti microbial) and (non pathogen? or nonpathogen?)
L39           1 FILE MEDLINE
L40           1 FILE CAPLUS
L41           4 FILE BIOSIS
L42           0 FILE EMBASE
L43           2 FILE WPIDS
L44           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L45           8 (ATIMICROBIAL OR ANTI MICROBIAL) AND (NON PATHOGEN? OR
NONPATHOG
                EN?)

=> s (antimicrobial or anti microbial or antibacterial agents/ct or
bactericide? or disinfectant? or antiseptic? or antibacterial agent)
L46       39089 FILE MEDLINE
L47      104937 FILE CAPLUS
L48       49576 FILE BIOSIS
L49       42549 FILE EMBASE
L50       39143 FILE WPIDS
L51       66257 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L52      341551 (ANTIMICROBIAL OR ANTI MICROBIAL OR ANTIBACTERIAL AGENTS/CT OR
                BACTERICIDE? OR DISINFECTANT? OR ANTISEPTIC? OR ANTIBACTERIAL
                AGENT)

=> s l52 and (escherchia or lactobacill? or lactococ? or bifidobacter? or
eubacter? or salmonella or propionobacter or shigella or campylobacter or
gardnerella or mycobacter? or pseudomonas or staphyloco?)
L53       10303 FILE MEDLINE
L54       21655 FILE CAPLUS
L55       22632 FILE BIOSIS
L56       12201 FILE EMBASE
L57        2860 FILE WPIDS
L58       10148 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L59       79799 L52 AND (ESCHERCHIA OR LACTOBACILL? OR LACTOCOC? OR
BIFIDOBACTER
                ? OR EUBACTER? OR SALMONELLA OR PROPIONOBACTER OR SHIGELLA OR
                CAMPYLOBACTER OR GARDNERELLA OR MYCOBACTER? OR PSEUDOMONAS OR
                STAPHYLOCO?)

=> s l59 and (plasmid? or rk2 or r6k or pcu1 or p15a or pip501 or pam(w)(b or
beta)(w)1 or pcrg1600 or rsf1010)
L60         427 FILE MEDLINE
L61         493 FILE CAPLUS
L62         814 FILE BIOSIS
L63         425 FILE EMBASE
L64          23 FILE WPIDS
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L65          160 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L66         2342 L59 AND (PLASMID? OR RK2 OR R6K OR PCU1 OR P15A OR PIP501 OR
                 PAM(W)(B OR BETA)(W) 1 OR PCRG1600 OR RSF1010)

=> s l66 and (replic? o duplic? or synthes?)
<------User Break------>
u
SEARCH ENDED BY USER
SEARCH ENDED BY USER

=> s l66 and (replic? or duplic? or synthes?)
L68           39 FILE MEDLINE
L69           54 FILE CAPLUS
L70           64 FILE BIOSIS
L71           44 FILE EMBASE
L72            6 FILE WPIDS
L73            8 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L74          215 L66 AND (REPLIC? OR DUPLIC? OR SYNTHES?)

=> s l74 and (transmiss? or donor bacterial or non pathogen?)
L75            2 FILE MEDLINE
L76            1 FILE CAPLUS
L77            1 FILE BIOSIS
L78            0 FILE EMBASE
L79            0 FILE WPIDS
L80            3 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L81            7 L74 AND (TRANSMISS? OR DONOR BACTERIAL OR NON PATHOGEN?)

=> dup rem l81
PROCESSING COMPLETED FOR L81
L82            6 DUP REM L81 (1 DUPLICATE REMOVED)

=> d cbib abs 1-6

L82  ANSWER 1 OF 6  BIOSIS  COPYRIGHT 2001 BIOSIS
2001:356055  Document No.: PREV200100356055. Importance of integrons in the
     diffusion of resistance. Carattoli, Alessandra (1). (1) Laboratory of
     Bacteriology and Medical Mycology, Istituto Superiore di Sanita, V.le
     Regina Elena 299, 00161, Rome: alecara@iss.it Italy. Veterinary Research
     (Paris), (May August, 2001) Vol. 32, No. 3-4, pp. 243-259. print. ISSN:
     0928-4249. Language: English. Summary Language: English; French.
AB   Horizontal transfer of resistance genes is a successful mechanism for the
     transmission and dissemination of multiple drug resistance among
     bacterial pathogens. The impact of horizontally transmitted genetic
     determinants in the evolution of resistance is particularly evident when
     resistance genes are physically associated in clusters and transferred en
     bloc to the recipient cell. Recent advances in the molecular
     characterisation of antibiotic resistance mechanisms have highlighted the
     existence of genetic structures, called integrons, involved in the
```

acquisition of resistance genes. These DNA elements have frequently been reported in multi-drug resistant strains isolated from animals and humans, and are located either on the bacterial chromosome or on broad-host-range **plasmids**. The role of integrons in the development of multiple resistance relies on their unique capacity to cluster and express drug resistance genes. Moreover, the spread of resistance genes among different **replicons** and their exchange between **plasmid** and bacterial chromosome are facilitated by the integration of integrons into transposable elements. The association of a highly efficient gene capture and expression system, together with the capacity for vertical and horizontal **transmission** of resistance genes represents a powerful weapon used by bacteria to combat the assault of antibiotics.

L82 ANSWER 2 OF 6 MEDLINE DUPLICATE 1
97333481 Document Number: 97333481. PubMed ID: 9189641. Molecular evolution of multiply-antibiotic-resistant **staphylococci**.
Skurray R A; Firth N. (School of Biological Sciences, University of Sydney, NSW, Australia.) CIBA FOUNDATION SYMPOSIUM, (1997) 207 167-83; discussion 183-91. Ref: 47. Journal code: D7X; 0356636. ISSN: 0300-5208. Pub. country: Netherlands. Language: English.

AB Methicillin-resistant **Staphylococcus** aureus (MRSA) is an intractable nosocomial pathogen. The chemotherapeutic intransigence of this organism stems from its predilection to **antimicrobial** resistance as a consequential response to selective pressures prevailing in the clinical environment. MRSA isolates are frequently resistant to all practicable **antimicrobials** except the glycopeptide, vancomycin. Although **antimicrobial** resistance sometimes arises via chromosomal mutation, the emergence of multiply-antibiotic-resistant **staphylococci** is primarily due to the acquisition of pre-existent resistance genes; such determinants can be encoded chromosomally or by **plasmids** and are often associated with transposons or insertion sequences. Clinical **staphylococci** commonly carry one or more **plasmids**, ranging from small **replicons** that are phenotypically cryptic or contain only a single resistance gene, to larger episomes that possess several such determinants and sometimes additionally encode systems that mediate their own conjugative **transmission** and the mobilization of other **plasmids**. The detection of closely related **plasmids**, elements and/or genes in other hosts, including coagulase-negative **staphylococci** and enterococci, attests to interspecific and intergeneric genetic exchange facilitated by mobile genetic elements and DNA transfer mechanisms. The extended genetic reservoir accessible to **staphylococci** afforded by such horizontal gene flux is fundamental to the acquisition, maintenance and dissemination of **staphylococcal antimicrobial** resistance in general, and multiresistance in particular.

L82 ANSWER 3 OF 6 JICST-EPlus COPYRIGHT 2001 JST
950898513 Recent trends of drug resistant mechanisms. Aminoglycoside group medicine. Resistant mechanisms of aminoglycoside antibiotics.. NISHINO TAKESHI. Kyoto Pharm. Univ.. Rinsho to Biseibutsu (Clinical

Microbiology). (1995) vol. 22, no. 5, pp. 535-541. Journal Code: F0933B (Fig. 4, Tbl. 1, Ref. 20) CODEN: 0910-7029; Pub. Country: Japan. Language:
Japanese.
AB The above chemical has many hydroxyl and amino groups for **antimicrobial** activity.Resistant bacteria, including Escherichia coli, **Pseudomonas**, and **staphylococci**, are known to produce enzymes that phosphorylate, adenylylate, or acetylate hydroxyl and
amino groups.Bacteria become resistant to the chemical by changing the ribosomal protein at the target site to decrease affinity with chemicals, or changing the permeability of the outer membrane.

L82 ANSWER 4 OF 6 JICST-EPlus COPYRIGHT 2001 JST
910888621 Novel **Plasmid** Vectors for Gene Cloning in **Pseudomonas**.. ITOH N; KOIDE Y; FUKUZAWA H; HIROSE S; INUKAI T. Fukui Univ., Fukui; Amano Pharmaceutical Co., Ltd., Aichi. J Biochem. (1991) vol. 110, no. 4, pp. 614-621. Journal Code: F0286A (Fig. 6, Tbl. 4,
Ref. 26) CODEN: JOBIAO; CODEN: 0021-924X; Pub. Country: Japan. Language: English.
AB Novel host-vector systems have been developed for gene cloning in the metabolically versatile bacterial genus **Pseudomonas**. We found that a new **Pseudomonas** strain, **Pseudomonas** flavida IF-4, isolated from soil, carried two small cryptic **plasmids**, named pNI10 and pNI20. They were multi-copy, but no self-**transmissible**, and the genome size was 3.7kb for pNI10 and 2.9kb for pNI20. Several types of cloning vectors containing a kanamycin or streptomycin resistance (Kmr or Smr) gene were constructed from pNI10 and pNI20. These **plasmid** vectors were efficiently transformed into several strains of **Pseudomonas** at a frequency up to 4*105 transformants per 1.MU.g **plasmid** DNA by the usual competent cell method. The vectors derived from pNI10 **replicated** not only in **Pseudomonas** but also in some other Gram-negative enteric bacteria such as Escherichia coli, Enterobacter aerogenes, and Proteus mirabilis. (author abst.)

L82 ANSWER 5 OF 6 JICST-EPlus COPYRIGHT 2001 JST
890498629 Mechanism of action of new quinolone drugs and mechanism of resistance.. HIRAI KEIJI. Kyorin Pharmaceutical Co., Ltd., Central Lab.. Chiryogaku (Biomedicine & Therapeutics). (1989) vol. 22, no. 6, pp. 589-593. Journal Code: Z0521B (Fig. 2, Tbl. 2, Ref. 15) CODEN: 0386-8109; Pub. Country: Japan. Language: Japanese.

L82 ANSWER 6 OF 6 MEDLINE
75102610 Document Number: 75102610. PubMed ID: 803707. R factors: **plasmids** conferring resistance to **antibacterial agents**. Stone A B. SCIENCE PROGRESS, (1975 Spring) 62 (245) 89-101. Journal code: UHR; 0411361. ISSN: 0036-8504. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Antibiotic sensitivity and resistance are often under the control of the bacterial chromosome. Frequently, however, an organism may exhibit resistance to one or several antibiotics as a dominant character determined by genes located on a **plasmid**, a relatively small, circular DNA molecule which **replicates**, with some degree of

autonomy, in the bacterial cytoplasm. Such **plasmids**, termed drug-resistance (R) factors, generally also specify the formation of sex pili, filamentous appendages on the cell surface. These promote bacterial conjugation, and hence permit the transfer of a copy of the **plasmid** from the resistant organism to one which may previously have been drug-sensitive. Each ex-conjugant is then capable of acting as a

plasmid donor during subsequent pairings, so that R factors are commonly responsible for the epidemic spread of multiple drug-resistance throughout an entire bacterial population. This can present serious problems in antibiotic therapy, particularly as **plasmids** are often **transmissible** between organisms of different species, and even different genera. The molecular nature, classification and behaviour of R factors is discussed.

```
=> s filutowicz m?/au,in
'IN' IS NOT A VALID FIELD CODE
L83          51 FILE MEDLINE
L84          53 FILE CAPLUS
L85          56 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L86          44 FILE EMBASE
L87           0 FILE WPIDS
L88           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L89         204 FILUTOWICZ M?/AU,IN

=> s l89 and l52
L90           0 FILE MEDLINE
L91           0 FILE CAPLUS
L92           0 FILE BIOSIS
L93           0 FILE EMBASE
L94           0 FILE WPIDS
L95           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L96           0 L89 AND L52

=> s l89 and (escherchia or lactobacill? or lactococ? or bifidobacter? or
eubacter? or salmonella or propionobacter or shigella or campylobacter or
gardnerella or mycobacter? or pseudomonas or staphyloco?)
L97           7 FILE MEDLINE
L98           7 FILE CAPLUS
L99          23 FILE BIOSIS
L100          7 FILE EMBASE
L101          0 FILE WPIDS
L102          0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L103         44 L89 AND (ESCHERCHIA OR LACTOBACILL? OR LACTOCOC? OR
BIFIDOBACTER
                ? OR EUBACTER? OR SALMONELLA OR PROPIONOBACTER OR SHIGELLA OR
                CAMPYLOBACTER OR GARDNERELLA OR MYCOBACTER? OR PSEUDOMONAS OR
```

STAPHYLOCO?)

=> s l103 and (transmiss? or replic? or duplic? or synthes? or non pathogen? or donor bacterial cell or marker gene or transfer gene)
L104 3 FILE MEDLINE
L105 2 FILE CAPLUS
L106 18 FILE BIOSIS
L107 3 FILE EMBASE
L108 0 FILE WPIDS
L109 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L110 26 L103 AND (TRANSMISS? OR REPLIC? OR DUPLIC? OR SYNTHES? OR NON PATHOGEN? OR DONOR BACTERIAL CELL OR MARKER GENE OR TRANSFER GENE)

=> s l110 and (plasmid? or rk2 or r6k or pcu1 or p15a or pip501 or pam(w)(b or beta)(w)1 or pcrg1600 or rsf1010)
L111 1 FILE MEDLINE
L112 1 FILE CAPLUS
L113 16 FILE BIOSIS
L114 1 FILE EMBASE
L115 0 FILE WPIDS
L116 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L117 19 L110 AND (PLASMID? OR RK2 OR R6K OR PCU1 OR P15A OR PIP501 OR PAM(W)(B OR BETA)(W) 1 OR PCRG1600 OR RSF1010)

=> s l117 not l81
L118 1 FILE MEDLINE
L119 1 FILE CAPLUS
L120 16 FILE BIOSIS
L121 1 FILE EMBASE
L122 0 FILE WPIDS
L123 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L124 19 L117 NOT L81

=> dup rem l124
PROCESSING COMPLETED FOR L124
L125 17 DUP REM L124 (2 DUPLICATES REMOVED)

=> d cbib abs 1-17

L125 ANSWER 1 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1998:473548 Document No.: PREV199800473548. **Replication** of **R6K** gamma origin in vitro: Discrete start sites for DNA **synthesis** dependent on pi and its copy-up variants. Chen, Dongzhao; Feng, Jin; Kruger, Ricardo; Urh, Marjeta; Inman, Ross B.; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol. Univ. Wisconsin, Madison, WI 53706 USA. Journal of Molecular Biology, (Oct. 2, 1998) Vol. 282, No. 4, pp. 775-787. ISSN: 0022-2836. Language: English.
AB The regulation of the **plasmid R6K** gamma origin (gamma

ori) is accomplished through the ability of the pi protein to act as an initiator and inhibitor of **replication**. Hyperactive variants of this protein, called copy-up pi, allow four to tenfold increases of gamma ori **plasmid** DNA in vivo. The higher activity of copy-up pi variants could be explained by an increase in the initiator function, a decrease in the inhibitor activity, or a derepression of a more efficient mechanism of **replication** that can be used by wt pi (pi35.0) only under certain conditions. We have compared the **replication** activities of Wt pi35.0 and copy-up pi mutants in vitro, and analyzed the **replication** pro- ducts. It is shown that copy-up variants are several-fold more active than wt pi35.0 in **replication**. This appears to be due to enhanced specific **replication** activity of copy-up mutants rather than elevated fractions of protein proficient in DNA binding. Furthermore, biochemical complementation revealed that pi200 (copy-up) is dominant over wt Tp35.0. The elevated activity of copy-up pi is not caused by an increased rate of replisome assembly as infer-red from in vitro **replication** assays in which the lag periods observed were similar to that of wt Tp35.0 Moreover, only one round of semiconservative, unidirectional **replication** occurred in all the samples analyzed indicating that copy-up pi proteins do not initiate multiple rounds of DNA **synthesis**. Rather, a larger fraction of DNA template **replicates** in the presence of copy-up pi as determined by electron microscopy. Two clusters of discrete DNA **synthesis** start sites are mapped by primer extension near the stability (stb) locus of the y ori. We show that the start sites are the same in the presence of wt pi35.0 or copy-up proteins. This comparative analysis suggests that wt pi35.0 and copy-up variants utilize fundamentally similar mechanism(s) of **replication** priming.

L125 ANSWER 2 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1998:510613 Document No.: PREV199800510613. Assemblies of **replication** initiator protein on symmetric and asymmetric DNA sequences depend on multiple protein oligomerization surfaces. Urh, Marjeta; Wu, Jianwei; Wu, Jiazhen; Forest, Katrina; Inman, Ross B.; **Filutowicz, Marcin (1)** . (1) Dep. Bacteriol., Univ. Wisconsin, Madison, WI 53706 USA. Journal of Molecular Biology, (Oct. 30, 1998) Vol. 28, No. 3, pp. 619-631. ISSN: 0022-2836. Language: English.

AB The pi35.0 protein of **plasmid R6K** regulates transcription and **replication** by binding a DNA sequence motif (TGAGR) arranged either asymmetrically into 22 bp direct repeats (DRs) in the gamma origin, or symmetrically into inverted half-repeats (IRs) in the operator of its own gene, pir. The binding patterns of the two natural forms of the pi protein and their heterodimers revealed that the predominant species, pi35.0 (35.0 kDa), can bind to a single copy of the DR as either a monomer or a dimer while tau30.5 (30.5 kDa) binds only as a dimer. We demonstrate that only one subunit of a pi35.0 dimer makes specific contact with DNA. Electron microscopic (EM) analysis of the nucleoprotein complexes formed by pi35.0 and DNA fragments containing all seven DRs revealed coupled ("hand-cuffed") DNA molecules that are aligned in a parallel orientation. Antiparallel orientations of the DNA were not observed. Thus, hand-cuffing depends on a highly ordered oligomerization of pi35.0 in such structures. The pi protein (pi35.0, pi35.0 binds to an

IR as a dimer or heterodimer but not as a monomer. Moreover, a single amino acid residue substitution, F200S (pir200), introduced into pi30.5 severely destabilizes dimers of this protein in solution and concomitantly prevents binding of this protein to the IR. This mutation also changes the stability of pi35.0 dimers but it does not change the ability of pi35.0 to bind IRs. To explain these observations we propose that the diverse interactions of tau variants with DNA are controlled by multiple surfaces for protein oligomerization.

L125 ANSWER 3 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1996:437477 Document No.: PREV199699151083. Preponderance of Fis-binding sites in the **R6K** gamma origin and the curious effect of the penicillin resistance marker on **replication** of this origin in the absence of Fis. Wu, Frank; Wu, Jiazhen; Ehley, Jennifer; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., E. B. Fred Hall, Univ. Wisconsin-Madison, 1550 Linden Dr., Madison, WI 53706 USA. Journal of Bacteriology, (1996) Vol. 178, No. 16, pp. 4965-4974. ISSN: 0021-9193. Language: English.

AB Fis protein is shown here to bind to 10 sites in the gamma origin of **plasmid** R6Y, The Fis-binding sites overlap all the previously identified binding sites in the gamma origin for the **plasmid** -encoded pi initiator protein and three host-encoded proteins, DnaA, integration host factor, and RNA polymerase. However, the requirement of Fis for **R6K replication** depends on the use of copy-up pi-protein variants and, oddly, the antibiotic resistance marker on the **plasmid**. In Fis-deficient cells, copy-up pi variants cannot drive **replication** of **R6K** gamma-origin **plasmids** carrying the bla gene encoding resistance to penicillin (Pen-r) but can drive **replication** of **plasmids** with the same origin but carrying the chloramphenicol acetyltransferase gene encoding chloramphenicol resistance (Cm-r). In contrast, **R6K replication** driven by wild-type pi is unaffected by the antibiotic resistance marker in the absence of Fis protein. Individually, none of these elements (copy-up pi, Fis deficiency, or drug markers) prevents **R6K replication**. The **replication** defect is not caused by penicillin in the medium or runaway **replication** and is unaffected by the orientation of the bla gene relative to the origin. **Replication** remains inhibited when part of the bla coding segment is deleted but the bla promoter is left intact. However, **replication** is restored by insertion of transcriptional terminators on either side of the gamma origin, suggesting that excess transcription from the bla gene may inactivate **replication** driven by pi copy-up mutants in the absence of Fis. This study suggests that vector sequences such as drug markers may not be inconsequential in **replication** studies, as is generally assumed.

L125 ANSWER 4 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1996:327315 Document No.: PREV199699049671. Initiator protein PI can bind independently to two domains of the gamma origin core of **plasmid R6K**: The direct repeats and the A+T-rich segment. Levchenko, Igor; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriology, Univ. Wis., Madison, WI 53706 USA. Nucleic Acids Research, (1996) Vol. 24, No. 10, pp.

1936-1942. ISSN: 0305-1048. Language: English.

AB The pi protein of **plasmid R6K** functions in both **replication** and transcription. pi autoregulates its own **synthesis** and is required for **replication** of the **R6K** gamma origin. pi performs these functions by binding to specific DNA sites arranged as pairs of 6-10 bp inverted repeats (IRs) or as a cluster of seven tandem 22 bp direct repeats (DRs) which lack symmetry. The sites share the TGAGRG nucleotide motif (where R is A or G).

The DRs and IRs flank the central A+T-rich segment of the gamma origin. In this work we carried out DNase I and hydroxyl radical protection experiments on various deletion derivatives of the gamma origin complexed with pi protein. These experiments revealed binding of pi to a novel site embedded within the A+T-rich segment. This interaction manifests primarily by the appearance of the enhanced scissions of DNA by DNase I and hydroxyl radicals. pi interaction with the A+T-rich site is independent of pi binding to the DRs and IRs. We propose that pi protein can recognize distinct families of DNA sequences in the gamma origin.

L125 ANSWER 5 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

1996:219185 Document No.: PREV199698775314. **Replication** of **plasmid R6K** gamma origin in vivo and in vitro: Dependence of IHF binding to the ihf1 site. Dellis, Stephanie; Feng, Jin; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., Univ. Wisconsin, 1550 Linden Dr., Madison, WI 53706 USA. Journal of Molecular Biology, (1996) Vol. 257, No. 3, pp. 550-560. ISSN: 0022-2836. Language: English.

AB The gamma origin of **plasmid R6K** requires the specific initiator protein pi for initiation of **replication**. However, increased pi concentrations inhibit **replication**. The host-encoded integration host factor (IHF) protein permits gamma origin **replication** at otherwise inhibitory pi levels. IHF is thought to mediate this positive effect by directly binding to the gamma origin. In this study we demonstrate that IHF binding to one IHF site in the gamma origin, ihf1, but not to the other side, ihf2, is necessary for the gamma origin to **replicate** at high pi protein levels. We also show that in vitro **replication** of the gamma origin **plasmid** requires IHF binding to the ihf1 site. Finally, we demonstrate both in vivo and in vitro that, when mutant pi proteins (hyperactive) are provided instead of wild-type pi, gamma origin **plasmids** can **replicate** in the absence of IHF. This supports a previously proposed hypothesis that the pi mutants can bypass the IHF requirement for gamma origin **replication**.

L125 ANSWER 6 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

1996:35053 Document No.: PREV199698607188. Altered (Copy-Up) forms of initiator protein pi suppress the point mutations inactivating the gamma origin of **plasmid R6K**. Urh, Marjeta; Flashner, Yehuda; Shafferman, Avigdor; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., Univ. Wisconsin, Madison, WI 53706 USA. Journal of Bacteriology, (1995) Vol. 177, No. 23, pp. 6732-6739. ISSN: 0021-9193.

Language: English.

AB The **R6K** gamma origin core contains the P2 promoter, whose -10 and -35 hexamers overlap two of the seven binding sites for the **R6K**-encoded pi protein. Two mutations, P2-201 and P2-203, which lie within the -35 region of P2, are shown to confer a promoter-down phenotype. We demonstrate here that these mutations prevent **replication** of a gamma origin core **plasmid**. To determine whether or not the reduced promoter activity caused by these mutations is responsible for their effect on **replication**, we generated two new mutations (P2-245-6-7 and P2-246) in the -10 hexamer of the P2 promoter. Although these new mutations inhibit P2 activity as much as the P2-201 and P2-203 mutations, they do not prevent **replication** of the gamma origin core. Therefore, activity of the P2 promoter does not appear to be required for **replication**. We also show that the inability of the gamma origin to function in the presence of the P2-201 and P2-203 mutations is reversed by the hyperactive variants of a protein called copy-up pi. This suppression occurs despite the fact that in vivo dimethyl sulfate methylation protection patterns of the gamma origin iterons are identical in cells producing wild-type pi and those producing copy-up pi variants. We discuss how the P2-201 and P2-203 mutations could inhibit **replication** of the gamma origin core and what mechanisms might allow the copy-up pi mutants to suppress this deficiency.

L125 ANSWER 7 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

1996:35003 Document No.: PREV199698607138. A DNA segment conferring stable maintenance on **R6K** gamma-origin core **replicons**. Wu, Frank; Levchenko, Igor; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., E. B. Fred Hall, Univ. Wisconsin-Madison, 1550 Linden Dr., Madison, WI 53706 USA. Journal of Bacteriology, (1995) Vol. 177, No. 22, pp. 6338-6345. ISSN: 0021-9193. Language: English.

AB The **plasmid R6K** gamma origin consists of two adjacent modules, the enhancer and the core, and requires **R6K** initiator protein pi for **replication**. While the core alone can **replicate** at a low level of wild-type pi protein, we show here that host cells do not stably maintain core **plasmids**. The presence of the enhancer segment confers stable inheritance on core **plasmids** without a significant change in average **plasmid** copy number. Deletions and site-directed mutagenesis indicated that the stability of core **plasmids** is not mediated by binding sites or consensus sequences in the enhancer for DnaA, Tr protein, gyrase, Fis, or Dcm methylase. Proper segregation of core **plasmids** requires only the **R6K** stb or stability-related region, which includes the 20-bp segment of the 100-bp enhancer adjacent to the core. The use of the pi-116 mutant protein, which increases **plasmid** copy number fourfold, does not stabilize core **plasmids** lacking the enhancer. We also show that at an elevated level of wild-type pi, the gamma-origin **plasmid** is unstable, even in the presence of the enhancer. We discuss the differences and similarities between the **R6K** stability system and those found in other **plasmids**.

L125 ANSWER 8 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

1995:543633 Document No.: PREV199698557933. Buffer composition mediates a switch between cooperative and independent binding of an initiator protein to DNA. Urh, Marjeta; York, Dona; **Filutowicz, Marcin (1)**. (1)

Dep. Bacteriol., Univ. Wisconsin-Madison, 1550 Linden Dr., Madison, WI 53706 USA. Gene (Amsterdam), (1995) Vol. 164, No. 1, pp. 1-7. ISSN: 0378-1119. Language: English.

AB The regulation of many biological processes, including DNA **replication**, is frequently achieved by protein-protein interactions, as well as protein-DNA interactions. Multiple protein-binding sites are often involved. For example, the **replication** of **plasmid R6K** involves binding of the initiator protein pi to seven 22-bp direct repeats (DR) in the gamma origin of **replication** (gamma ori). A mutant protein pi-S87N has been isolated, that in Tris-borate buffer (TB) binds cooperatively to seven DR, whereas wild-type (wt) pi binds independently (Filutowicz et al., Nucleic Acids Res. 22 (1994) 4211-4215). Surprisingly, we found that wt pi can also bind cooperatively when Tris cntdot acetate (TA), Tris cntdot succinate or Tris cntdot glutamate buffers are used instead of TB. The cooperative binding of the wt pi protein was also observed in the TB buffer at high concentrations of Na-2EDTA. These results suggest that pi may be able to assume two functionally distinct conformations as a result of either mutation or buffer composition. Moreover, we found that the mode of pi binding is determined not by the composition of the buffer in which the reaction was assembled, but by the composition of the electrophoresis buffer. We discuss the general implications of these findings.

L125 ANSWER 9 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

1994:459341 Document No.: PREV199497472341. Regulation of **replication** of an iteron-containing DNA molecule. **Filutowicz, Marcin (1)**; Dellis, Stephanie; Levchenko, Igor; Urh, Marjeta; Wu, Frank; York, Dona. (1) Dep. Bacteriol., Uiniv. Wis.-Madison, Madison, WI 53706 USA. Cohn, W. E. [Editor]; Moldave, K. [Editor]. Progress in Nucleic Acid Research and Molecular Biology, (1994) Vol. 48, pp. 239-273. Progress in Nucleic Acid Research and Molecular Biology. Publisher: Academic Press, Inc. 1250 Sixth Ave., San Diego, California 92101, USA. ISSN: 0079-6603. ISBN: 0-12-540048-9. Language: English.

L125 ANSWER 10 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

1995:20145 Document No.: PREV199598034445. Binding of DnaA protein to a **replication** enhancer counteracts the inhibition of **plasmid R6K** gamma origin **replication** mediated by elevated levels of **R6K** pi protein. Wu, Frank; Levchenko, Igor; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., E.B. Fred Hall, Univ. Wis.-Madison, 1550 Linden Drive, Madison, WI 53706 USA. Journal of Bacteriology, (1994) Vol. 176, No. 22, pp. 6795-6801. ISSN: 0021-9193. Language: English.

AB **Replication** of the gamma origin of Escherichia coli **plasmid R6K** requires pi protein, encoded by the **R6K** pir gene, and many host factors, including DnaA protein. pi has dual roles, activating **replication** at low levels and inhibiting **replication** at high levels. The inhibitory function of pi is counteracted by integration host factor and a specific sequence of the origin called the enhancer. This 106-bp DNA segment contains a binding site for DnaA protein (DnaA box 1). In this study, we mutated this site to determine if it was required for the enhancer's function. Using gamma origin derivative **plasmids** with the DnaA box 1 altered or

deleted, we show that this site is necessary to protect the origin against the levels of wild-type pi protein that would otherwise inhibit **replication**. To show that the base substitutions in DnaA box 1 weakened the binding of DnaA, we developed a new application of the agarose gel retardation assay. This quick and easy assay has broad applicability, as shown in binding studies with DNA fragments carrying a different segment of the **R6K** origin, the chromosomal origin (oriC), or the pUC origin. The gel retardation assay suggests a stoichiometry of DnaA binding different from that deduced from other assays.

L125 ANSWER 11 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1995:26219 Document No.: PREV199598040519. Cooperative binding of initiator protein to **replication** origin conferred by single amino acid substitution. **Filutowicz, Marcin (1)**; York, Dona; Levchenko, Igor. (1) Dep. Bacteriol., Univ. Wisconsin, Madison, WI 53706 USA. Nucleic Acids Research, (1994) Vol. 22, No. 20, pp. 4211-4215. ISSN: 0305-1048. Language: English.

AB The **replication** initiator protein pi of **plasmid R6K** binds seven 22 bp direct repeats (DR) in the gamma origin. The pi protein also binds to an inverted repeat (IR) in the operator of its own gene, pir, which lies outside the gamma origin sequences. A genetic system was devised to select for pi protein mutants which discriminate between IR and DR (York et al., Gene (Amst.) 116, 7 - 12, 1992; York and Filutowicz, J. Biol. Chem. 268, 21854-21861, 1993). From this selection the mutant pi-S87N protein was isolated which is deficient in repressing the pir gene's expression because it cannot bind to IR at the pir gene operator. Remarkably, we discovered that pi-S87N binds to DR cooperatively under conditions where wt pi binds independently. Moreover, the pi-S87N is more active as a **replication** initiator in vivo when supplied at the same level as wt pi. Quantitative binding assays showed that both wt pi and pi-S87N bind a DNA fragment containing a single DR unit with a similar affinity (K-d = 0.3 times 10-12 M). Thus, cooperativity of pi-S87N is most likely achieved through altered interactions between protomers bound at adjacent DR units.

L125 ANSWER 12 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1994:390194 Document No.: PREV199497403194. In vivo excision and amplification of large segments of the Escherichia coli genome. Posfai, Gyorgy; Knoob, Michael; Hradecna, Zdenka; Hasan, Noaman; **Filutowicz, Marcin**; Szybalski, Waclaw (1). (1) McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI 53706 USA. Nucleic Acids Research, (1994) Vol. 22, No. 12, pp. 2392-2398. ISSN: 0305-1048. Language: English.

AB In vivo excision and amplification of large segments of a genome offer an alternative to heterologous DNA cloning. By obtaining predetermined fragments of the chromosome directly from the original organism, the problems of clone stability and clone identification are alleviated. This approach involves the insertion of two recognition sequences for a

site-specific recombinase into the genome at predetermined sites, 50 - 100
kb apart. The integration of these sequences, together with a conditional **replication** origin (ori), is targeted by homologous recombination. The strain carrying the insertions is stably maintained until, upon induction of specifically engineered genes, the host cell expresses the site-specific recombinase and an ori-specific **replication** protein. The recombinase then excises and circularizes the genomic segment
flanked by the two insertions. This excised DNA, which contains ori, is amplified with the aid of the **replication** protein and can be isolated as a large **plasmid**. The feasibility of such an approach is demonstrated here for E. coli. Using the yeast FLP/FRT site-specific recombination system and the pi/gamma-ori **replication** initiation of **plasmid R6K**, we have devised a procedure that should allow the isolation of virtually any segment of the E. coli genome.
This was shown by excising, amplifying and isolating the 51-kb lacZ-phoB and the 110-kb dapX-dsdC region of the E. coli MG1655 genome.

L125 ANSWER 13 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1994:435256 Document No.: PREV199497448256. The dimerization domain of **R6K plasmid replication** initiator protein sigma revealed by analysis of a truncated protein. Levchenko, Igor; York, Dona; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., Univ. Wisconsin, Madison, WI 53706 USA. Gene (Amsterdam), (1994) Vol. 145, No. 1, pp. 65-68. ISSN: 0378-1119. Language: English.

AB **Replication** of **plasmid R6K** is controlled by the homodimeric initiator protein pi, which binds to seven 22-bp direct repeats (iterons) in the gamma-origin. One of the genetically engineered pi variants (DELTA-C164-pi) contains only the 164 N-terminal amino acids (aa) of the 305-aa pi molecule. This truncated pi polypeptide retains the ability to function as a specific inhibitor of **R6K replication** in vivo, though it neither drives **replication**, nor binds to iterons (Greener et al., Mol. Gen. Genet. 224 (1990) 24-32). In order to define the region of pi responsible for dimerization, we have performed chemical crosslinking experiments with purified DELTA-C164-pi and shown that this polypeptide is dimeric. We did not observe an exchange between protein monomers upon mixing of wild-type pi and DELTA-C164-pi homodimers. However, heterodimers, as well as each type of homodimers, were formed when these polypeptides refolded after guanidine hydrochloride treatment. Thus, both dimerization and dimer stability are determined by the N-terminal domain of pi. We speculate that
these properties might depend on the leucine zipper and RGD motifs that have been identified in the two regions of the N-terminal domain of pi.

L125 ANSWER 14 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1993:581547 Document No.: PREV199497000917. Autoregulation-deficient mutant of
the **plasmid R6K**-encoded pi protein distinguishes between palindromic and nonpalindromic binding sites. York, Dona; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriology, E.B. Fred Hall, 1550 Linden Dr., Univ. Wisconsin, Madison, WI 53706 USA. Journal of Biological Chemistry, (1993) Vol. 268, No. 29, pp. 21854-21861. ISSN:

0021-9258. Language: English.

AB The autogenously regulated gene pir of Escherichia coli **plasmid R6K** encodes the **replication** protein pi. This protein binds to two sites in the operator region of the pir gene: a 22-base pair nonpalindromic sequence and a pair of palindromic 9-base pair sequences. These pi-binding sites are similar, suggesting that pi uses a single DNA-binding domain in recognizing them. We devised a **plasmid** system permitting isolation of mutants of the pi protein which are altered in autoregulation. A Ser-87 to Asn substitution in one such mutant, designated pi-87, reduces the protein's ability to repress the pir gene promoter in vivo. DNase I protection and gel retardation assays were carried out with highly purified pi-87 protein. In these studies pi-87 exhibited altered binding to the palindromic but not to the nonpalindromic part of the operator of the pir gene. Chemical cross-linking and gel filtration analyses have shown that the dimerization properties of wild type pi and pi-87 proteins are similar in solution. We propose that the interaction of pi protein with the palindromic part of the pir operator is essential for autoregulation; we also propose that there is a fundamental difference in the mechanisms of pi protein recognition of palindromic and nonpalindromic sequences.

L125 ANSWER 15 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

1993:145770 Document No.: PREV199395078570. Two alternative structures can be formed by IHF protein binding to the **plasmid R6K** gamma origin. Dellis, Stephanie; Schatz, Todd; Rutlin, Kay; Inman, Ross B.; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., Univ. Wis., Madison, Wis. 53706 USA. Journal of Biological Chemistry, (1992) Vol. 267, No. 34, pp. 24426-24432. ISSN: 0021-9258. Language: English.

AB Escherichia coli integration host factor (IHF) contributes to the regulation of **R6K plasmid** copy number by counteracting the inhibitory activity of the **plasmid**-encoded **replication** protein pi. Two IHF-binding sites (ihf1 and ihf2) flank seven iterons in the origin which bind pi protein. As previously shown by electron microscopy, IHF can compact a large segment of the **R6K** gamma origin DNA, encompassing site ihf1, an AT-rich domain containing ihf1, and some of the seven iterons located downstream of ihf1. We termed this phenomenon IHF-mediated DNA folding. This folding requires a high IHF concentration, and the region of the origin (**replication** enhancer) located to the left of the AT-rich domain. However, site ihf2 is not necessary in forming the folded structure. As reported here, IHF binding to ihf2 can be detected in gel mobility shift assays only if the leftmost enhancer region is absent. Sites ihf1 and ihf2 each contain two consensus IHF sequences. Site-directed mutagenesis was performed to determine which sequences are recognized by IHF protein and which sites are involved in forming the various gamma origin-IHF complexes. Finally, we define the boundaries of protection from DNaseI digestion when IHF is bound to ihf2. We propose a model in which IHF protein bound to ihf1, in the absence of the enhancer region, facilitates IHF binding to ihf2.

L125 ANSWER 16 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
1994:435199 Document No.: PREV199497448199. Translational options for the pir gene of **plasmid R6K**: Multiple forms of the **replication** initiator protein pi. York, Dona; Ivanov, Vladimir; Gan, Jacek; **Filutowicz, Marcin (1)**. (1) Dep. Bacteriol., 1550 Linden Drive, Univ. Wis., Madison, WI 53706 USA. Gene (Amsterdam), (1992) Vol. 116, No. 1, pp. 7-12. ISSN: 0378-1119. Language: English.

AB The autogenously controlled pir gene of **plasmid R6K** was believed to encode a single polypeptide that plays multiple roles in the **plasmid**'s biology. We have isolated an opal (op) mutant at the 18th codon of the pir coding frame which does not totally abolish translation of pir mRNA. In extracts of cells containing this mutation two translational products (35 kDa and 30.2 kDa) have been detected. We propose that the 35-kDa polypeptide produced by the pir 18 op mutant contains Trp substituted for Arg-18 as the result of an opal readthrough. Translation, which results int he 30.2-kDa polypeptide, originated downstream from the UGA stop signal created by the mutation. Moreover, we realize now that the 30.2-kDa polypeptide is also produced in cells containing a wild-type (wt) pir gene. The shorter variant on the pi protein lacks **replication** initiation and inhibition functions, as well as autorepressor activity in vivo. We also show that an in-frame fusion of seven N-terminal codons of the trpE gene with a pir gene lacking the first two codons produces two polypeptides which replace the 35-kDa pi protein and are of similar molecular weight. Thus, at least three options exist in the translation of the wt pir mRNA. Start codons are most likely at codon positions 1, 6 or 7 and 36 or 38. Each of these five AUG codons is preceded by a consensus ribosome-binding site (RBS).

L125 ANSWER 17 OF 17 MEDLINE DUPLICATE 1
83247799 Document Number: 83247799. PubMed ID: 6346358.
Replication of derivatives of the broad host range **plasmid RK2** in two distantly related bacteria. Schmidhauser T J; **Filutowicz M**; Helinski D R. PLASMID, (1983 May) 9 (3) 325-30. Journal code: P8P; 7802221. ISSN: 0147-619X. Pub. country: United States. Language: English.

AB A 0.7-kb segment of the broad host range **plasmid RK2** containing the **replication** origin of this **plasmid** will **replicate** in Escherichia coli and **Pseudomonas** putida when this segment is joined to a 1.8-kb region of **RK2** designated traA*. The presence of another region of **RK2**, designated trfB, that previously was implicated in **RK2 replication** had no effect on the maintenance of the **RK2** trfA*-oriV **replicon** in these two organisms. These observations indicate a requirement for a minimal account of information for **replication** of this broad host range **plasmid** in two distantly related bacteria.

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Prepared by M. Hale 308-4258